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Pathology, molecular genetics, and epigenetics of diffuse intrinsic pontine glioma

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Diffuse intrinsic pontine glioma (DIPG) is a devastating pediatric brain cancer with no effective therapy. Histological similarity of DIPG to supratentorial high-grade astrocytomas of adults has led to assumptions that these entities possess similar underlying molecular properties and therefore similar therapeutic responses to standard therapies. The failure of all clinical trials in the last 30 years to improve DIPG patient outcome has suggested otherwise. Recent studies employing next-generation sequencing and microarray technologies have provided a breadth of evidence highlighting the unique molecular genetics and epigenetics of this cancer, distinguishing it from both adult and pediatric cerebral high-grade astrocytomas. This review describes the most common molecular genetic and epigenetic signatures of DIPG in the context of molecular subgroups and histopathological diagnosis, including this tumor entity's unique mutational landscape, copy number alterations, and structural variants, as well as epigenetic changes on the global DNA and histone levels. The increased knowledge of DIPG biology and histopathology has opened doors to new diagnostic and therapeutic avenues.

Keywords: DIPG, glioma, glioblastoma, pediatric, ACVR1, K27M, histone, H3F3A

Introduction

Diffuse intrinsic pontine gliomas (DIPG), brainstem tumors that diffusely involve the pons, are the most common type of brainstem gliomas (BSG) (1). The mean age of diagnosis for this devastating pediatric neoplasm is 6–7 years (2, 3). The delicate location of these tumors eliminates surgical intervention as a treatment option. Radiation therapy (RT) is the standard of care, although it offers temporary relief of symptoms rather than a real hope of cure (4). Despite 68 clinical trials using various adjuvant chemotherapeutic agents between 1984 and 2014, there has been no improvement in survival compared to radiation alone, and DIPG are currently the number one cause of brain tumor related death in children (5–18). The median survival of DIPG patients is only 10 months post diagnosis and <10% of patients survive 2 years (19, 20). Since the 1980s, the diagnosis of DIPG was based on clinical findings and diagnostic imaging characteristics on computerized tomography (CT) or magnetic resonance imaging (MRI) (4, 21). The lack of surgical and biopsy material has limited most studies of DIPG biology and histology to post-mortem tissue. Although initial investigation into the safety of incorporating biopsy for BSG showed no surgical mortality and low surgical morbidity (22), the advent of CT and MRI allowed for accurate non-invasive localization of BSG (23). It was noted that patients with DIPG represented the majority of deaths in children with BSG and biopsies, which did not stratify patients into different treatment groups, were abandoned (23).

Recently, the role for biopsy in DIPG diagnosis has been substantially strengthened due to several important discoveries related to the biology and histopathology of this universally fatal tumor (3, 24, 25) and successful use of stereotactic biopsy by several centers with limited morbidity (26–29). Biologically, DIPG is a unique tumor entity which possesses properties that are antithetical when compared to other brain tumors, such as pediatric and adult supratentorial high-grade astrocytomas (HGA), which they most often resemble histologically. This review highlights the unique pathology, genetics, and epigenetics of DIPG.

From Candidate Gene Approaches to Next-Generation Sequencing

Early molecular profiling of pediatric gliomas was limited to candidate gene approaches (30–33) focusing on mutational and copy number profiling of genes known to be frequently implicated in adult HGA, namely *EGFR*, *TP53*, *IDH1/2*, *CDKN2A*, *MGMT*, and *PTEN* (34–36). All early studies of DIPG were performed on small cohorts or case studies, which limited their usefulness in defining the biology of this deadly pediatric cancer. However, conclusions from these studies still highlighted some differences between DIPG and adult HGA. Unfortunately, these candidate gene approaches were limited to what was known about adult HGA and future studies using whole-genome profiling approaches would be required to discover more robust differences between these entities. Whole genome profiling technologies such as array CGH and SNP-genotyping allowed for the first genome-wide copy number analysis of cancers. Although giving a low resolution perspective at first, newer iterations allowed for greater and greater resolution and began to highlight the unique molecular profiles of DIPG when compared to pediatric and adult supratentorial astrocytomas. Copy number signatures at the whole chromosome arm level revealed differences between DIPG and adult HGA (37). Gains of chromosome 1q and losses of 11p, 13q, and 14q were frequently observed in DIPG and less so in supratentorial HGA (37). Subsequent copy number studies, with larger DIPG patient tumor cohorts, revealed frequently altered genes (**Table 1**) including *PDGFRA*, *TP53, PARP1, PVT-1/MYC*, *RB1*, and *PTEN* (3, 38, 39). Most recently, next-generation sequencing approaches, including whole-genome sequencing (WGS), whole-exome sequencing (WES), and RNA-sequencing integrated with histopathology, copy number, gene expression, and methylation profiling, and other molecular techniques have re-defined what we know about DIPG genetics and epigenetics.

TABLE 1 | Frequencies of most common mutations and copy number alterations in diffuse intrinsic pontine glioma from the literature.

Gene	Alteration type	Frequency in DIPG (%)	Reference
ACVR1	Mutation	20–32	(40–42)
ATRX	Mutation	9–13	(3, 24)
BRAF	Mutation	0	(40–43)
CDK6	Copy number gain	3–4	(3, 37, 44)
CDKN2A/B	Focal deletion	3–4	(37–39)
EGFR	Mutation	0–2	(40–43, 45)
	Amplification	0–2	
FGFR	Fusion	0	(40–43)
H3F3A	Mutation	58–65	(3, 40–43, 46)
HIST1H3B/C	Mutation	12–19	(40–43, 46)
IDH1/2	Mutation	0	(3, 40–43, 46)
MYC/PVT-1	Copy number gain	14	(3, 40)
MYCN	Copy number gain	7	(3, 40)
NF1	Mutation	0–3	(40–43)
	Focal deletion	7	
PDGFRA	Mutation	5–9	(3, 37, 38, 40, 47, 48)
	Copy number gain	28–36	
PIK3CA	Mutation	12–23	(2, 27)
PPM1D	Mutation	10–12	(42, 46)
TP53	Mutation	42–71	(2, 3, 32, 40, 49)
	Heterozygous deletion	35–64	
PTEN	Mutation	0–3	
	Focal deletion	14	(38–43)
RB1	Mutation	0	(3, 38–43)
	Focal deletion	16	

Mutational Landscape

The first major breakthrough in defining the DIPG mutational landscape came in 2012, when studies on pediatric brain tumors using whole-genome and WES reported that 70–84% of DIPG possess mutations in histone H3, and that these mutations were predictive of outcome (3, 24, 25). These recurrent mutations, in *H3F3A* or *HIST1H3B/C/I* (**Figure 1A**), result in a p. Lys27Met (K27M) substitution. A subset of other midline astrocytomas, such as those arising in the thalamus, have also been found to harbor K27M histone H3 mutations although at a lower frequency (50). Conversely, pediatric supratentorial HGA rarely possess these mutations, and more frequently have p. Gly34Arg (G34R) or p. Gly34Val (G34V) substitution in histone H3.3. The G34R/V-H3.3 mutations occur in 10–19% of supratentorial HGA cases (24) and are never found in DIPG (3). Furthermore, among supratentorial GBM, these G34R/V-H3.3 mutations are predominantly found in older children and young adults (3). Mutations affecting these two histone residues are extremely rare in adult HGA.

Histones are proteins that form octomeric complexes known as nucleosomes around which DNA wraps and condenses into chromatin. The majority of histone mutations in DIPG, 65%, effect histone variant H3.3 (3, 24, 25). Although this histone is coded in two genes, *H3F3A* and *H3F3B*, K27M mutations are only found in the former. K27M-H3.1 mutations are found in 12–19% of DIPG and are mutually exclusive from K27M-H3.3 mutations (24, 40). Histone H3.1 is coded by a cluster of 10 genes on chromosome 6 known as the *HIST1* cluster (44). Currently, K27M-H3.1 mutations have been detected in *HIST1H3B*, *HIST1H3C*, and *HISTIH3I*.

Histones play a key role in the state of chromatin; however, they themselves do not determine whether chromatin will be in a conformation more permissive to gene expression (euchromatin) or in a conformation less permissive to gene expression (heterochromatin). Several histone marks can be laid on specific residues across the histone tail, including lysine 27, which can be post-translationally modified by either acetylation or mono-, di-, or tri- methylation (51–53). Various cellular machineries are implicated in the reading, writing, and copying of these epigenetic histone marks, including histone acetyltransferases (HATs), histone

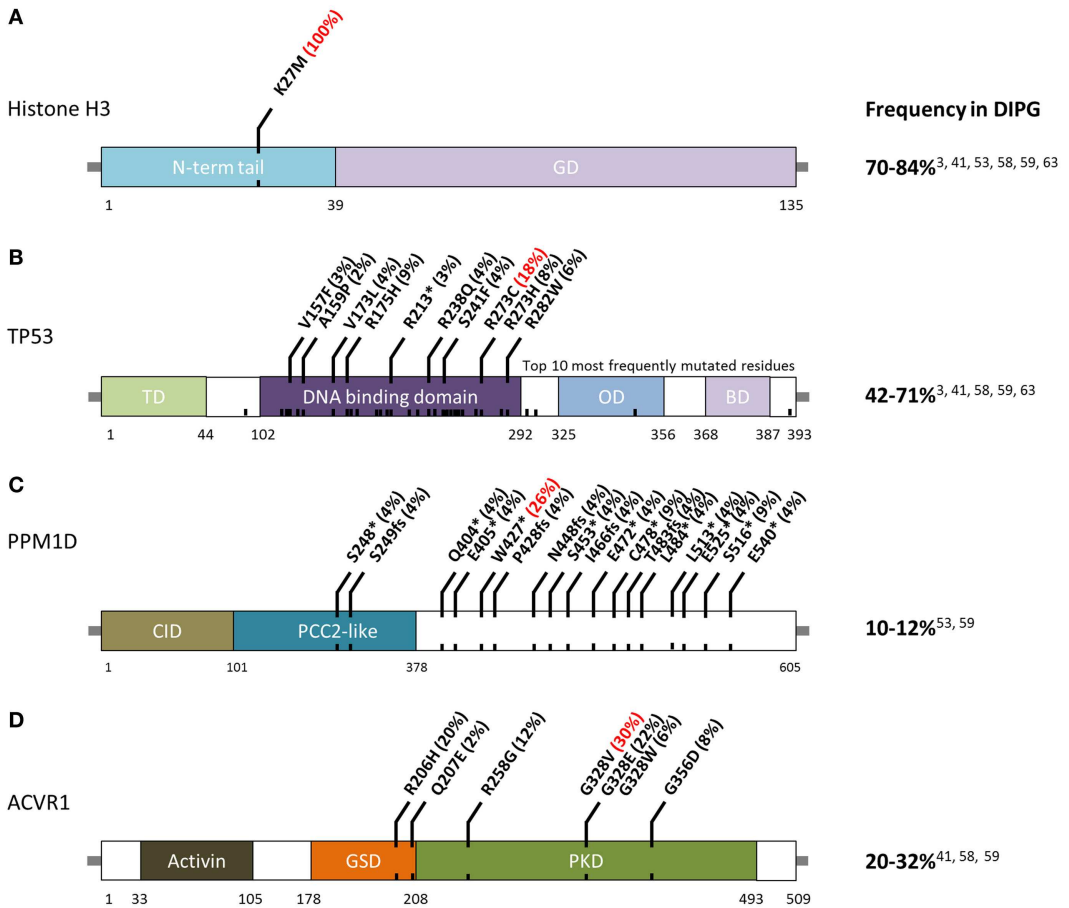

FIGURE 1 | Schematic of residue alterations in (A) histone H3, (B) TP53, (C) PPM1D, and (D) ACVR1 as a result of mutations most frequently identified in DIPG. GD, globular domain; TD, transactivation domain; OD, oligomerization domain; BD, basic domain; CID, CHEK1 interacting domain; GSD, GS domain; PKD, protein Kinase domain. Red indicates most frequently altered amino acid.

deacetylases (HDACs), histone methyltransferases (HMTs), and histone demethylases (54). The methionine substitution at K27 in DIPG cannot be modified and several *in vitro* and *in vivo* studies have documented a global decrease in H3K27me3 in the presence of mutant histone (40, 55–58). This global decrease is attributed to polycomb repressive complex 2 (PRC2) inhibition (59); however, other molecular consequences of this mutation, including its effect on histone marks, global DNA methylation, and gene expression, are still under investigation. Histone H3 mutations are undoubtedly important in DIPG tumorigenesis and/or maintenance but in a majority of cases are found to be partnered with other mutations and copy number alterations (CNAs) (3, 40), suggesting that they are not the sole drivers of DIPG tumorigenesis.

TP53 mutations and CNAs occur in 42–71% of DIPG (**Figure 1B**) (3, 40). Hemizygous deletions are often associated with a mutation and strong protein expression by immunohistochemistry (32, 49). Interestingly, DIPG with low-grade astrocytoma histology (grade II), even those harboring H3-mutations, are not mutated for *TP53* (2). Among DIPG with high-grade histology (grade III and IV), both H3-mutant and wild-type DIPG often have mutated *TP53* (2). However, a recent exome sequencing study identified *PPM1D* mutations (**Figure 1C**) in a subset of H3-mutant but *TP53* wild-type DIPG (46). These mutations account of ~50% of *TP53* wild-type grade II DIPG. *PPM1D* gene codes for WIP1 (wild-type p53-induced protein phosphatase 1D) and has been implicated as an oncogene in other cancers (60–62). Mutations of *PPM1D* have been shown to be functionally equivalent to those of *TP53* (46, 63). Taken together, alterations of the *TP53* signaling pathway in DIPG are only slightly less common than histone H3 mutations.

The third most commonly mutated gene in DIPG codes for the activin A receptor, type I (*ACVR1*), a member of the bone morphogenic protein (BMP) signaling pathway. Approximately, 20–32% of DIPG harbor mutations in *ACVR1* which significantly overlap with K27M mutations in histone H3.1 (40–42). Previously only reported in a congenital autosomal dominant disease of the connective tissue called fibrodysplasia ossificans progressiva (FOP), *ACVR1* mutations result in ligand-independent constitutive activation of the BMP signaling pathway (64–66). Although seven different *ACVR1* mutations have been reported in DIPG, the most common alteration in this tumor type, p.Gly328Val, has not been reported in FOP patients. Several other residues of *ACVR1* are frequently mutated in DIPG (**Figure 1D**). These mutations have been shown to increase levels of phosphorylated SMAD1/5

(40–43) as well as increased gene expression of downstream BMP signaling targets *ID1* and *ID2* (40). Research into the cooperation between *ACVR1* and histone H3.1 mutations, and their effects on tumorigenesis are still ongoing.

Amplification and Mutations of Receptor Tyrosine Kinases

Receptor tyrosine kinases (RTKs) are cell surface receptors which are often dysregulated in cancers. The RTK/RAS/PI-3K signaling pathway is the most commonly dysregulated signaling pathway in adult GBM, with 90% of tumors exhibiting CNAs or mutations in pathway members (34). Mutations and CNAs in the platelet-derived growth factor receptor alpha (*PDGFRA*) have been implicated in both adult and pediatric HGA. Several array based studies of DIPG revealed amplification of *PDGFRA* in 28–36% of patient tumors (37, 38). The frequency of these amplifications in pediatric and adult supratentorial HGA was reported in the range of 7–14%, and was preferentially identified in the Proneural subtype of adult GBM (36). In DIPG, *PDGFRA* amplifications are exclusively found in patients with K27M-H3 mutations and across all astrocytic histologies (grade II-IV) (2, 40). Oncogenic mutations of *PDGFRA* have also been reported in 5–9% of DIPG (47, 48). Epidermal growth factor receptor (*EGFR*) copy number gains and mutations are among the most frequent alterations in adult HGA, occurring in 60–85% of GBM (34, 35). In adults, EGFR protein expression correlates with amplifications and EGFRvIII mutations; however in DIPG, *EGFR* CNAs and mutations are extremely rare, found in 0–2% of patient tumors (45), and do not correlate with immunopositivity (67, 68). The RTK/RAS/PI-3K signaling pathway is further dysregulated in DIPG through hemizygous deletions of *PTEN* (40), as well as mutations of *PIK3CA* and *PIK3R1* (2, 27). Although this pathway has often been targeted in clinical trials, the majority of the CNAs and mutations in the RTK/RAS/PI-3K pathway are clonal events, as determined by fluorescence *in situ* hybridization (FISH) for *PDGFRA* amplifications (37) and mutation allele frequency of *PIK3CA*, suggesting these events likely arise later in tumor development.

DNA Damage Repair (PARP1, MGMT, MPG)

Radiotherapy is the standard of care for DIPG and adjuvant chemotherapeutics have been shown to be ineffective. Perturbations in DNA damage repair pathways in DIPG were first identified in 2010 by Zarghooni et al. Loss of heterozygosity (LOH) was identified in many genes involved in nucleotide excision repair, non-homologous end-joining (NHEJ), homologous recombination (HR), base excision repair (BER), and mismatch repair (MMR) by analysis of both SNP arrays and microsatellite markers (37). Poly (ADP-ribose) polymerase (PARP1), a protein essential for repair of single strand DNA breaks induced by alkylating agents, as well as repair of ionizing radiation induced double strand breaks by HR and NHEJ were found to be gained and/or overexpressed in 54% of DIPG, highlighting it as a potential therapeutic target (37). Furthermore, pathway analysis revealed a subset of patients with LOH or deletions in members of the *BRCA* DNA damage response pathway, including *BRCA1* and *BRCA2*. Defects in either of these two genes have been implicated in promoting sensitivity to single strand DNA repair via PARP inhibition.

Temozolomide (TMZ) is a frontline DNA alkylating agent most often used in treatment of adult GBM. TMZ causes DNA damage by alkylating O^6-guanine, N^7-guanine, and N^3-adenine residues. In adults, it was identified that a subgroup of patients with *MGMT* (O6-methylguanine DNA methyltransferase) promoter methylation had improved overall survival when treated with radiotherapy and concomitant TMZ compared to patients without *MGMT* promoter methylation (69). MGMT repairs TMZ alkylated O6-guanine nucleotides. However, the universal lack of response to TMZ in DIPG patients could not be attributed to this resistance mechanism, as DIPG have not been found to express MGMT (37). It has been recently described that TMZ resistance in the pediatric population can be attributed to an ATM-dependent regulation of 3-methylpurine-DNA glycosylase (MPG), an enzyme responsible for repair of alkylated N^7 guanine and N^3 adenine residues (70). Further investigation into using radio-sensitizing and chemo-sensitizing agents in DIPG therapy is warranted.

Isocitrate Dehydrogenase, *ATRX*, and Telomeres

Mutations in the mitochondrial enzyme gene, isocitrate dehydrogenase 1 (*IDH1*), whose gene product catalyzes the oxidative decarboxylation of isocitrate to α-keto glutarate, are found in approximately 70–80% of adult low-grade astrocytomas (LGA) and anaplastic astrocytomas (AA), as well as secondary glioblastoma (GBM) (71). Furthermore, in adult gliomas, *IDH1* mutations were found to be associated with *ATRX* mutations and alternative lengthening of telomeres (ALT) (72). Isocitrate dehydrogenase 2 (*IDH2*) mutations are also found in a subset of adult HGA but to date, no mutations in *IDH1* or *IDH2* have been detected in DIPG.

Mutations of chromatin remodeling genes are less common in DIPG than in supratentorial HGA (3, 24). *ATRX*, which codes for the α-thalassemia/mental retardation syndrome X-linked gene, was found to be mutated in a subset of DIPG (~9%) but in contrast to pediatric supratentorial HGA, had no clear overlap with histone H3 mutations (3). Pediatric supratentorial HGA showed high overlap with *ATRX* or *DAXX* mutations (15–25%) and G34R/V-H3.3 alterations, as well as mutual exclusivity of *IDH1/2* mutations (24, 73). Irrespective of tumor location, in the pediatric population *ATRX* mutations significantly overlap with *TP53* mutations and are predominantly found in older children (3). *ATRX* and *DAXX* (death-domain associated protein) are genes encoding subunits of a chromatin remodeling complex required for histone H3.3 incorporation at telomeric regions. Telomeres are repetitive regions of DNA found on the ends of chromosomes and shorten during every cell division due to incomplete DNA replication (74). Over many cell divisions, telomeres may reach a critically short length, which results in cellular senescence (75). This fate can be avoided by expressing telomerase, an enzyme that can extend telomeres. Although telomerase is not expressed in most mature, terminally differentiated cells (76, 77), its expression and activity has been implicated in several brain cancers as a poor prognostic marker and potential therapeutic target (78–80). Extension of telomeres

in certain cancers can be attained by a telomerase independent mechanism known as alternative lengthening of telomeres (ALT). Twenty percent of DIPG test positive for ALT by either TRF (telomere restriction fragment) assay or C-circles assay and ALT positive DIPG are exclusive carriers of the K27M-H3.3 mutation (40). ALT phenotype is also associated with an older age of diagnosis in DIPG and to date has not been detected in patients with low-grade astrocytoma histology (2, 40).

DIPG Histopathology

Diffuse intrinsic pontine glioma is a heterogeneous disease, and represents a varied histological spectrum. These tumors are very diffuse and often involve adjacent brain structures beyond the pons. Several studies report leptomeningeal dissemination and subventricular spread as a common occurrence seen in as many as one-third of DIPG, with tumor cells found as far rostrally as the frontal lobe (2, 81, 82). A review by Jansen et al. reported World Health Organization (WHO) central nervous system tumor classification on 108 biopsies from 13 studies, including 37 AA (WHO grade III), 27 GBM(WHO grade IV), 22 LGA (WHO grade II), 3 anaplastic oligoastrocytomas (WHO grade III), and 19 tumors with "not further specified" or undefined characterization (9). Data from this biopsy series would suggest that WHO grade III AA are the most common histological entity in brainstem glioma; however, autopsy based histopathological studies report WHO grade IV GBM to be the most common histology, potentially highlighting the caveat of limited tissue sampling during biopsy or anaplastic progress and/or treatment effect seen in late stage disease at autopsy. Autopsy based studies revealed that GBM histology was most common in DIPG. Of the 33 pediatric patients examined by Yoshimura et al., 29 were reported to be GBM and 4 with anaplastic astrocytoma histology (83). A larger autopsy based study of DIPG histology reported 42 GBM, 18 anaplastic astrocytoma, 8 low-grade astrocytoma, and 2 with features of primitive neuroectodermal tumor (PNET, WHO grade IV) (13).

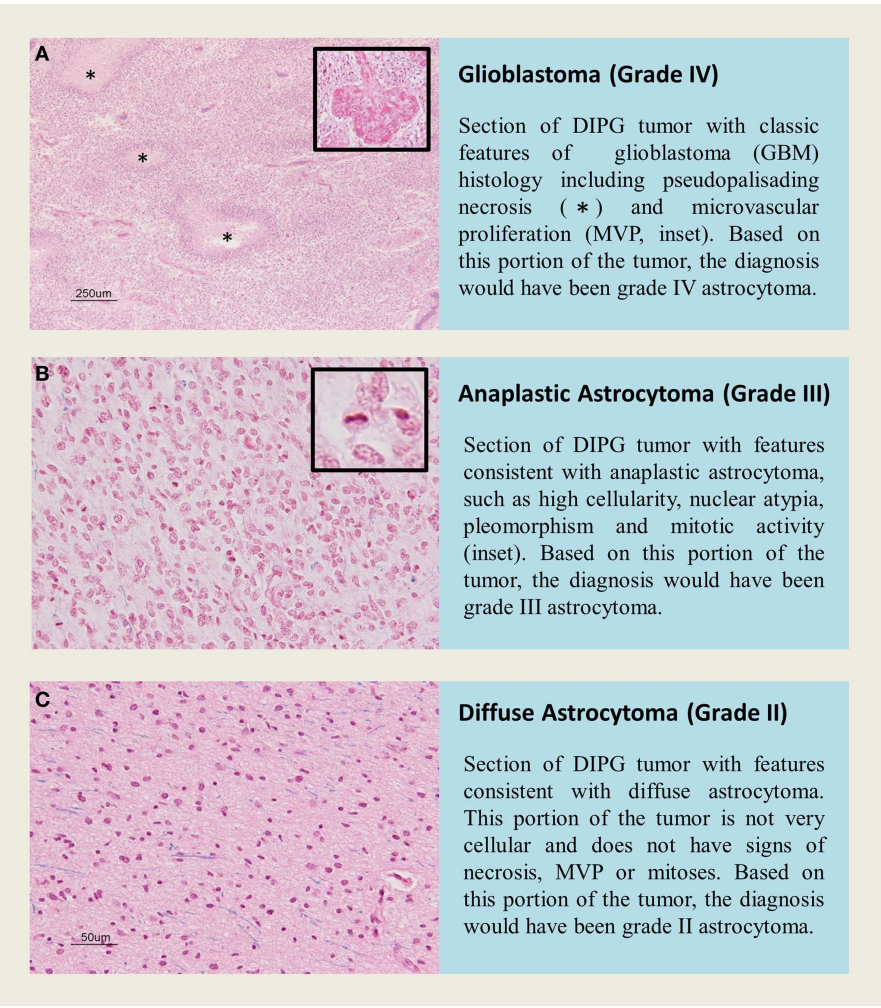

FIGURE 2 | H&E staining of tissue sections from an autopsy of a K27M-H3.1 mutant diffuse intrinsic pontine glioma patient highlights vast regional differences that histologically resemble (A,B) high grade astrocytoma (WHO grade III–IV) or (C) low grade astrocytoma (WHO grade II). Inadvertently targeting these regions on biopsy could lead to misdiagnosis. Histone H3 mutation predicts overall survival better than histologic grade for DIPG. Mutational testing at the time of stereotactic biopsy should be implemented into clinical practice for DIPG patients.

Previous investigations into DIPG histology have also observed this rare PNET histology in the brainstem (84). Importantly, regional differences within one DIPG specimen can bias biopsy based diagnoses. Autopsy studies allow extensive tissue sampling and have highlighted intratumoral histopathologic heterogeneity (2). Areas within or around a grade IV astrocytoma may present with features of grade II or grade III histology that could be inadvertently targeted at biopsy (**Figure 2**).

Perhaps most importantly, DIPG histology is not a predictor of survival. Patients with low-grade histology do just as poorly as patients with high-grade histology. On multivariate Cox regression analysis, only histone H3 mutation is a predictor of worse

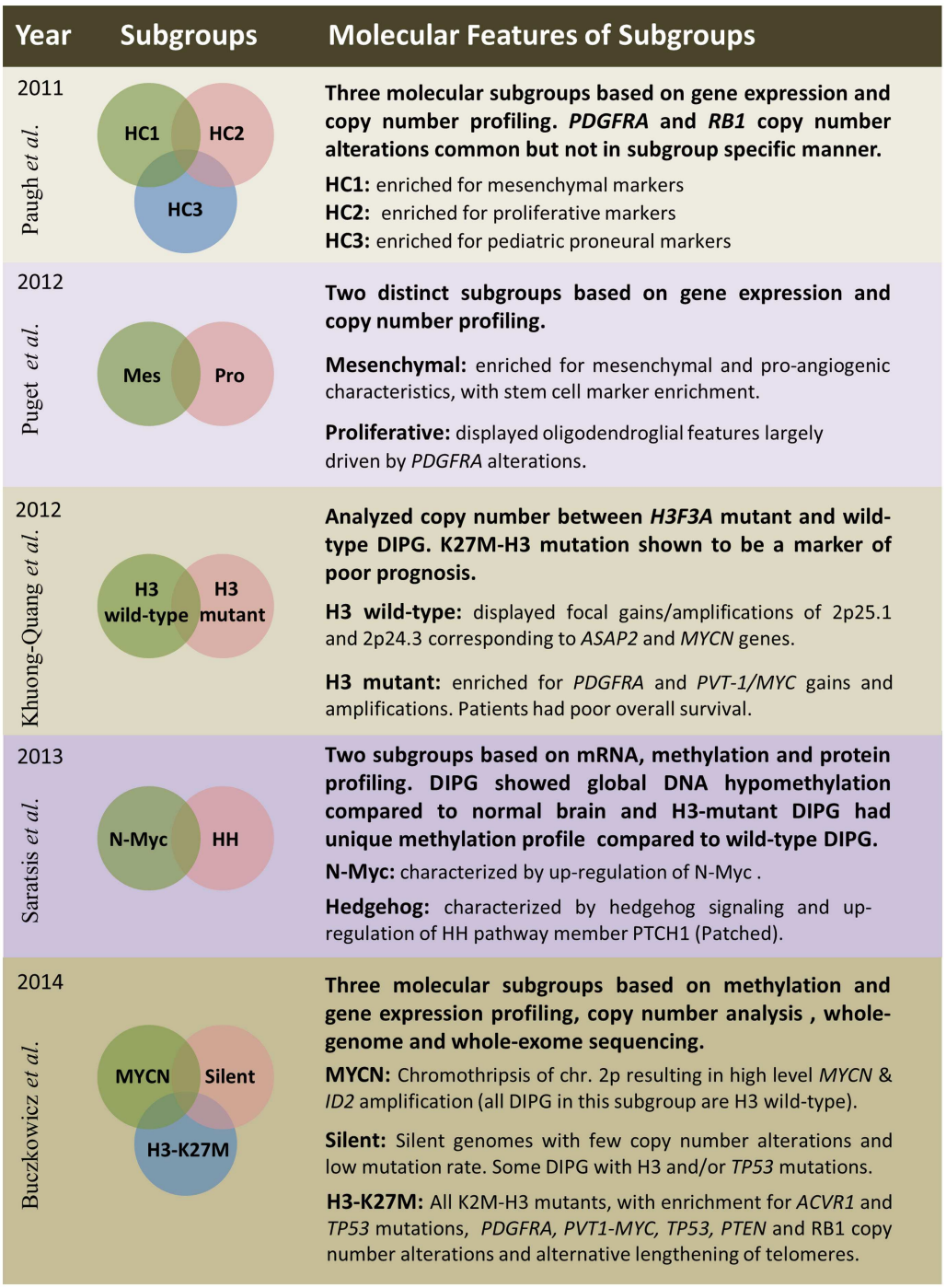

FIGURE 3 | Summary of five publications between 2011 and 2014 outlining clinical and molecular features of diffuse intrinsic pontine gliomas based on various platforms and sample sizes; Paugh et al. (38), three subgroups – $n = 27$; Puget et al. (48), two subgroups – $n = 23$; Khuong-Quang et al. (3), two subgroups – $n = 42$; Saratsis et al. (85), two subgroups – $n = 14$; Buczkowicz et al. (40), three subgroups – $n = 48$.

overall survival, irrespective of histological grade, age of diagnosis, and sex (2, 3). Furthermore, DIPG patients, which at autopsy had grade II histology, but were mutated for histone H3, had clinical outcomes similar to what would be expected of high-grade tumors (2, 3). This important finding suggests brainstem glioma require their own WHO grading scheme, which needs to incorporate H3-mutation testing at biopsy.

Molecular Subgroups of DIPG

Several studies have embarked on molecular subgrouping of DIPG based on various molecular signatures, including gene expression profiling, copy number analysis, proteomics, mutational profiles, and methylation profiling (**Figure 3**). A study by Paugh et al. in 2011 profiled 27 DIPG by gene expression arrays and classified them into three subgroups based on hierarchical clustering and compared the subgroup specific enrichment scores to previously identified subgroups of adult GBM, highlighting significant enrichment in either mesenchymal, proliferative, or pronerual markers (38). These data were also compared to genomic copy number abnormalities determined by single-nucleotide polymorphism arrays which highlighted frequent focal amplifications of *PDGFRA* and *RB1*. However, these CNAs were not restricted to any of the three identified subgroups. A subsequent study of gene expression profiles from 23 DIPG matched with copy number data from array CGH identified two subgroups using K-means clustering (48). These two subgroups were enriched for mesenchymal and proneural markers, respectively, with the proneural subgroup displaying oligodendroglial features and alterations of *PDGFRA* (48). With the discovery of histone mutations in ~80% of DIPG in 2012 and their clinical relevance in patient survival, supervised analysis of CNAs in wild-type and histone mutant subgroups by single-nucleotide polymorphism arrays highlighted *PDGFRA* and *PVT-1/MYC* gains and amplifications to be present at high frequency in K27M-H3 mutant DIPG, whereas wild-type DIPG were enriched for *MYCN* amplification as determined by GISTIC2.0 analysis (3). A study from 2013 by Saratsis et al. utilized mRNA and methylation profiles with protein profiling of 14 DIPG specimens. This study also reported two subgroups, which were characterized by upregulation of *N*-Myc or Hedgehog signaling through mRNA expression and DNA hypomethylation (85).

The most comprehensive subgrouping of DIPG integrated CpG island methylation with WGS and WES, gene expression, and copy number profiling to find that DIPG are three molecularly distinct subgroups; MYCN, Silent, and H3-K27M (40). The MYCN subgroup did not contain any DIPG with histone mutations, but was characterized by high-level amplifications of *MYCN* and *ID2* genes caused by chromothripsis on chromosome 2p. Furthermore, these *MYCN* amplified DIPG did not contain *PVT-1/MYC*, *PDGFRA,* or *RB1* CNAs, which were previously described to be common in these brainstem neoplasms (40). The Silent subgroup did not contain many CNAs or mutations. In fact, this subgroup was characterized by stable genomes when compared to both MYCN and H3-K27M subgroup DIPG. Some DIPG in this subgroup contained histone H3, *TP53*, and *ACVR1* mutations; however, they were found at a lower frequency. All patients within the largest subgroup, H3-K27M, had histone H3-mutations. This subgroup was further characterized by hypomethylated genomes and also enriched in *ACVR1* mutations (which significantly co-occurred with K27M-H3.1 mutations), *TP53/PPM1D* mutations/homozygous deletions, alternative lengthening of telomeres, and CNAs of *PVT-1/MYC*, *PTEN*, *PDGFRA*, and *RB1* (40).

Conclusion

In recent years, the combined increase in knowledge of DIPG histology, genetics, and epigenetics has been substantial. Mutations in previously undiscovered oncogenes, histone H3, and *ACVR1*, molecular subtypes of DIPG, and the rediscovered role for biopsy for this tumor entity are redefining what is clinically possible in treating these patients. The major challenges ahead are developing molecularly accurate pre-clinical models for testing of new therapeutics and integrating new genetic/epigenetic and histopathological knowledge for DIPG patient diagnosis, treatment, and future clinical trial design. In the near future, DIPG patients will benefit from mutational testing and molecular subgrouping at biopsy, which will provide more accurate prognosis and actionable tumor-specific genomic targets for these affected children.

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